COAL SULFUR TRANSFORMATIONS MONITORED
BY HYPERTHERMOPHILIC ARCHAEBACTERIA

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Introduction

Microbial processes have been used as the basis for significant chemical transformations in several industries including but not limited to pharmaceuticals, foods processing and waste management. However, biotransformations are often limited by the metabolic rate of the organism and the stability of its constituent enzymes and other biomolecules. Industries which have successfully applied microorganisms for commercial purposes have developed processes that are consistent with whatever biological limitations prevail.

The spread of biotechnology has resulted in considerable attention to the use of biological systems in mineral and fossil fuel processing. For example, the acidophilic bacterium *Thiobacillus ferrooxidans* has been used in copper and uranium leaching as well as in small scale studies of pyrite removal from coal [1,2]. Other potential uses for microbes in coal processing include desulfurization, denitrification, oxygen removal, solubilization and gasification of coals [3]. The wide range of metabolic characteristics available in the global pool of microorganisms may enable researchers to discern more about the functionalities in coal structure as well as to build cultures in which the desired conversions can be engineered.

Hyperthermophiles

During the past decade, several bacteria have been isolated that thrive at temperatures at or above $100\,^\circ C$ [4]. These hyperthermophiles may have potential for a variety of significant biotransformations in the field of biotechnology. The high temperature optima of hyperthermophiles and their associated biomolecules may be useful in industrial processes by carrying out transformations at faster rates and with high levels of stability. Recently, we have been evaluating the potential for using hyperthermophilic archaebacteria in coal upgrading [5]. In doing so, both the ecology and physiology of these organisms must be considered.

Hyperthermophiles are associated with geothermally heated areas, which often are sulfur- and metal-rich environments. Several sulfur-metabolizing species have been isolated from these areas. The ability of these organisms to transform sulfur compounds at high temperatures is the interesting metabolic trait that could be capitalized upon for sulfur removal from coal. However, the nature of the various sulfur metabolisms differ and are not completely understood [4].

In preliminary studies, *Pyrococcus furiosus*, an anaerobic heterotroph isolated from geothermally heated marine sediments off of the coast of Vulcano, Italy [6], has been used as a representative hyperthermophilic strain. In the absence of S^0, *P. furiosus*, produces H_2, which is inhibitory for growth, and CO_2 [7,8]. In the presence of S^0, *P. furiosus* produces H_2S, either respiring sulfur or utilizing it to remove H_2 from the environment [8]. *P. furiosus* grows to higher maximum cell densities (10^8 cells/ml) with faster doubling times (1 hour) than most of the hyperthermophilic

archaebacteria (6,9). Because of the easier cultivation of the organism, *P. furiosus* appears to be more suitable for coal upgrading. In addition to sulfur removal capabilty, thermophilic heterotrophy may make *P. furiosus* suitable for breaking down complex coal constituents.

Hyperthermophiles and coal-sulfur

Previously, we examined the feasibility of coal desulfurization with *P. furiosus* (5,10,11). Various coals and model compounds were screened for sulfur removal activity. Success in sulfur removal was measured by the production of H_2S. Only highly weathered gob coals showed the production of H_2S upon exposure to *P. furiosus* (5,11). Continuous culture of *P. furiosus* with coal containing elemental sulfur showed that *P. furiosus* can remove sulfur from coal at rates comparable and in most cases higher than mesophilic organisms studied for sulfur removal (10). The drawback is that the sulfur within coal must be in the form useful to the microorganism in order to be converted. Very few of the more pristine coals tested showed H_2S production upon exposure to *P. furiosus*, suggesting that sulfur metabolizable by *P. furiosus* is not present in these coals (5,11).

Model compound studies showed that P. furiosus metabolizes only those sulfur compounds which are polysulfidic or form polysulfides near culture temperatures (98°C). Compounds with $-(S-S-S)-_x$ bonds are believed to be only a small fraction of coal sulfur. The specificity of *P. furiosus* for these compounds may be useful in settling a few disputes about polysulfidic compound generation in coal. (7)

Coal Weathering and Sulfur Speciation

Elemental sulfur in coal is believed to be a product of pyrite oxidation (12-14). During coal weathering under varying reaction conditions, oxidation products from FeS_2 have been reported to include S_n as well as FeO, FeS, Fe_2O_3, $FeSO_4$, $Fe_2(SO_4)_3$, SO_2 and SO_3. The weathering products of the organic sulfur components of coal have not yet been determined, because the organic sulfur species themselves are not known. Postulated organic sulfur compounds in coal are thiols, sulfides, disulfides, and thiophenic residues. Oxidation experiments both with model sulfur species and with coal have resulted in the production of sulfones, sulfonic acids and sulfates (12,13).

The formation of elemental sulfur from organic sulfur compounds under mild oxidation is thought to be unlikely primarily because the oxidation of the organic moieties would require gasification of the carbon skeleton of the coal (14). Mild oxidation of coals is a surface phenomenon involving the addition of oxygen molecules and small-scale rearrangement. Breakdown of the carbon backbone would require more extreme processing conditions (15).

By ASTM guidelines, sulfur speciation in coals requires experimental determination of sulfatic, pyritic, and total sulfur content. Organic sulfur is determined by difference. This calculational procedure tends to propagate experimental error of the analytical techniques and therefore leads to inaccuracy in organic sulfur values (12,13,16-18). In addition to technical error, the presence of elemental sulfur can lead to overestimation of organic sulfur content (19).

Artificial Weathering Experiments

Many mild oxidation experiments have been run on on both coal and mineral pyrite in attempts to discern the mechanisms of sulfur transformations during weathering. Although the reactivities differ between ore pyrite and coal pyrites and among pyrites from different coal sources (20-22), parameters that have been tested and shown to affect the distribution of sulfur oxidation products for all pyritic substrates include: temperature, humidity, oxygen content, acidity and the presence of chemical oxidants.

Water, both in vapor and liquid forms has been shown to have an accelerating effect on the weathering process (12,13,17,24). These results indicate the interaction of water with oxygen groups on the coal surface. The enhancement of oxygen uptake due to the presence of water agrees with the theory of peroxygen formation at the coal surface as the initiation step of weathering (25).

Results from several artificial weathering studies suggest that the mechanism of pyrite oxidation is strongly dependent upon temperature. Sulfate products have been found to prevail at low temperature conditions, while a complex range of oxidation products are generated at higher temperatures (13,17). Even within the range of 'realistic' weathering temperatures (25 to 80°C), the products of coal pyrite oxidation vary considerably. (23)

In the pyrite crystal, iron is anchored in the lattice and sulfurs extend from the surface. Each of the two pyritic sulfurs is bound to another sulfur molecule yeilding an FeS_4^{-4} configuration of pyrite. The extended sulfurs become oxygenated as a result of weathering. Oxygenation can occur as the result of peroxygen or hydrated Fe^{3+} producing dissociation conditions on the pyrite surface. (26) The Fe-S bond weakens and breaks before the S-S disulfide groups. This is confirmed by the appearance of thiosulfate as a specific intermediate (26,27). The production of a polysulfide could be consistent with this theory. At pH lower than 5 thiosulfate decomposes to elemental sulfur and sulfate.

There have been several studies of pyrite oxidation in aqueous suspension. Most of the work at low temperature 30°C and pH greater than 6 has shown little or no production of elemental sulfur. McKay and Halpern weathered mineral pyrite in aqueous suspension under varying levels of acidity (27). Through mass balance it was suggested that elemental sulfur was formed at low levels. These authors assumed that no thiosulfates and thionates were formed from the oxidation. All of the oxidized sulfur that was not accounted for in the form of sulfate was assumed to be elemental sulfur. It was found that 'elemental' sulfur was produced at low pH.

Moses et. al. used ion chromatography to analyze pyrite oxidation solutions for sulfoxy anions (26). At higher pH, oxidation of pyrite to sulfate was rapid with little production of sulfoxy intermediates. At low pH, 10 - 25% of sulfur in solution was determined to be thiosulfate and polythionate. No analysis for elemental sulfur was performed.

Like Moses, Goldhaber predicts the production of elemental sulfur at low pH. However, neither of these researchers performed low pH oxidations (28). McKibben and Barnes oxidized pyrite at low temperature under acidic conditions and did not note the production of elemental sulfur (29).

Despite agreement on the theory of the formation of sulfoxy intermediates, their is some confusion as to the products of pyrite oxidation. Luther suggests that in the presence of excess Fe^{3+}, thiosulfate is oxidized to sulfate (26). Meyer used Fe^{3+} to oxidize pyrite and produced elemental sulfur as an oxidation product (30). Which parameters can be manipulated to increase the selectivity of the thiosulfate oxidation for elemental sulfur has yet to be determined.

The microbial role in weathering

The deposition of elemental sulfur in coals is thought to be largely the result of microbial action (31). Although most of the microbial generation of elemntal sulfur is thought to be through sulfate reduction, several pyrite oxidizing organisms have been reported to produced S^0. Numerous studies on the feasibility of microbial coal desulfurization have focused on the use of such organisms for leaching pyrite from coals. Much of the work has centered around acidophilic aerobic systems including *Thiobacillus ferrooxidans, Sulfolobus*, and mixed cultures of *T. ferrooxidans* and *T. thiooxidans* (1-3). These bacteria mediate the oxidation of FeS_2 to Fe^{3+} and $SO_4^=$. Elemental sulfur has frequently been observed as an intermediate or end product of these oxidations (32).

Due to the aerobic nature of microbial pyrite leaching systems, it is difficult to discern whether the distribution of oxidation products is a direct result of microbial metabolism or of simultaneous abiotic weathering. It has been suggested that the initial dissolution of FeS_2 to Fe^{2+} is a result of chemical weathering (33). Microbial action mediates the oxidation of Fe^{2+} to Fe^{3+} at rates higher than those in the absence of microbial catalysts (34) The accelerated accumulation of Fe^{3+} in the biological systems could account for the production of more elemental sulfur than in chemical weathering. In this scenario, elemental sulfur is not the result of direct microbial action, but of the precipitation of sulfur in the acidic culture media as affected by Fe^{3+} levels in solution. However, it is plausible that elemental sulfur is generated by the microbiological utilization of

pyritic sulfur.

Pyrite leaching experiments with a *Leptospirrilum*-like bacterium in pure culture produced higher levels of elemental sulfur than in abiotic weathering (34) . In mixed culture with *T. ferrooxidans* sulfur yields were comparable if not lower than abiotic controls. These results suggest that the *Leptospirrilum*-like organism "prefers" the presence of S^0 whereas S^0 is oxidized by *T. ferroozidans*. The discussion becomes more confusing as the severity of the weathering conditions are considered. The *Leptospirrilum*-like bacterium carries out pyrite leaching reactions at pH values lower than *T. ferrooxidans*. The higher acidity of the culture environment may be the determining factor in S^0 generation. Again, the interaction between biological and abiotic sulfur production is unclear and may vary from organism to organism.

Experimental

Currently, we are investigating the effects of varying conditions on the products of pyrite weathering. Motivations for this study are two-fold: 1.) to achieve a chemical/microbial mechanism that would enable us to capitalize on the high rates of S^0 or plysulfide utilization of *P. furiosus* 2.) to determine the involvement of both organic and inorgainic sulfur species in the deposition of polysulfidic compounds in coal.

Using approaches outlined in previous literature on artifical weathering, we are examining the activity of *P. furiosus* to sulfur species in coal exposed to various levels of temperature, acidity, ferric iron concentrations among other parameters. Weathering conditions have been chosen in attempt to optimize the selectivity for reducible sulfur generation rather than sulfate formation. In conjuntion with the *P. furiosus* bioassay, ASTM speciation of coal sulfur, as well as standard chemical analysis for sulfoxy intermediates in solution are being used to follow the transformation of sulfur species during weathering.

Figure 1 shows the results of a long-term coal weathering experiment in which *P. furiosus* is used to determine the levels of available reducible sulfur. The results illustrate the interrelationship between iron and sulfur species and show that the reducible sulfur availble to the bacteria varies through the process. The weathering conditions are relatively mild so that only inorganic sulfur in coal is likely to be affected. The bacteria in the bioassay are probably active towards elemental sulfur formed from pyrite. The sulfide generated at varous time points suggest that there is no accumulation of S^0 but that it is an intermediate in the weathering process as inorganic sulfur is converted to sulfate.

The exposure of coals to elevated levels of oxygen and temrprature under aqueous conditions has been considered as a means for both inorganic and organic sulfur removal from coal. Air/water oxydesulfurization of coal was evaluated by Warzinski et. al. (35) They showed that while inorganic sulfur could be converted to sulfate under conditions that minimized the loss in heating value of coals, organic sulfur removal lead to significant heating value losses. For example, for an Indiana No.5 coal, they showed that the percent heating value loss in the coal was approimately the same as the apparent sulfur removal. However it may be possible that more subtle changes in the coal matrizx related to certain sulur moietis cold lead to effective biological treatment.

Table 1 shows the results of a mild weathering experiment using two coals: a pristime Illinois No. 6 containing 0.7% (w/w) pyritc sulfur and 3.4% (w/w) organic sulfur; and an Australian brown coal containing 0.03% (w/w) pyritic sulfur and 3.7% (w/w) organic sulfur. After 21 days of exposure to the conditions listed, coal samples were incubated with *P. furiosus* at 98 ˚C to determine to what extent the coal sulfur had become available to theis bacterium. Only an end point sample of weathered coal was taken in this weathering experiment. Whether the distribution of sulfur endproducts is the result of one day of weathering versus 21 days of weathering cannot be ascertained. Note the unweathered coals yilded little if any sulfide when exposed to *P. furiosus*. In several of the experiments with the Australian coal, we detected significantly larger amounts of sulfide than in the experiments with the Illinois coal. Although only about 1% of the organic sulur in the Australian coal was apparently converted to sulfide, the fact that less sulfide was generted from the Illinois coal was surprising. The pyritic sulfur in the Illinois coal was expected to be converted to elemental sulfur and sulfate with the result that significant amounts of sulfide would be

generated from S^0 by *P. furiosus*. The differences between the sulfide generated form the two coals may or may not reflect differences in tbe forms of orgnaic sulfur in the two coals. As mentioned earlier, *P. fuiosus* metabolizes aliphatic sulfur compounds such as cystine (8) but not aromatic sulfur in compounds such as dibenzothiophene. Further work is need in the development of the bioassy, but it is clear tbat biological activity may prove to be a sensitive and illuminating probe for sulfur speciation in coals.

Summary

In developing biologically-based desulfurization process to determine not only the organic sulfur content of the coal, but how it occurs. For example, certain microbial systems will be more active to aliphatic sulfur than aromatic sulfur. While analytical techniques for determining the distribution of organic sulfur in coal are emerging, they are not readily available. Nonetheless, microbial treatment processes based on better chance of succeeding. Secondly, organic sulfur removal in coal may be best approached by a combination of biological and chemical steps. The chemical steps need not severely reduce the heating value of tbe coal but rather be directed at modifying the sulfur bound in the coal matrix to improve its biological availability. For the case of the weathering experiments described here, it would be interesting to see if microbially mediated weathering of coal would produce similar changes in sulfur availability to sulfur reducers such as *P. furiosus*. This would suggest that the microbial portion of a cbemical/mirobial treatment system not be limeited to a particular species, but may encompass several microbial metabolisms. The weathering experiment described here is prelimenary, but closer examination to combining the oxydesulfurization process with subsequent biological treatment will be persued.

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| Coal	Temp (°C)	pH	Air (atm)	Sulfate (ppm)	Sulfide (nmol/ml)	Cell Density (cells/ml)
Table 1. Artificial Weathering Experiments with Illinois/Australian Coals						
Sulfide Generation by _Pyrococcus furiosus_						
AUS	80	1.6	1	953	21	9.8E6
	80	0.6	1	1480	16	1.5E7
	80	1.6	3	280	160	1.4E7
	80	0.6	3	275	263	1.1E7
	100	1.6	1	262	133	9.9E6
	100	0.6	1	967	158	1.3E7
	100	1.6	3	250	89	9.0E6
	100	0.6	3	358	152	1.0E7
ILL	80	1.6	1	738	22	8.8E6
	80	1.6	1	870	25	8.8E6
	80	1.6	1	798	20	9.4E6
				±54	±2	±0.2E6
	80	0.6	1	1015	63	1.5E7
	80	1.6	3	798	93	1.1E7
	80	0.6	3	1743	67	8.3E6
	100	1.6	1	228	74	1.5E7
	100	0.6	1	645	18	1.2E7
	100	1.6	3	905	45	1.0E7
	100	0.6	3	824	51	1.1E7
P. furiosus Growth on S°					>2000	2.4E8
AUS (Unweathered)					30	8.4E6
ILL (Unweathered)					12	9.6E6

Weathering of Illinois #6
(70 C, pH 1.6, 1Atm Air)
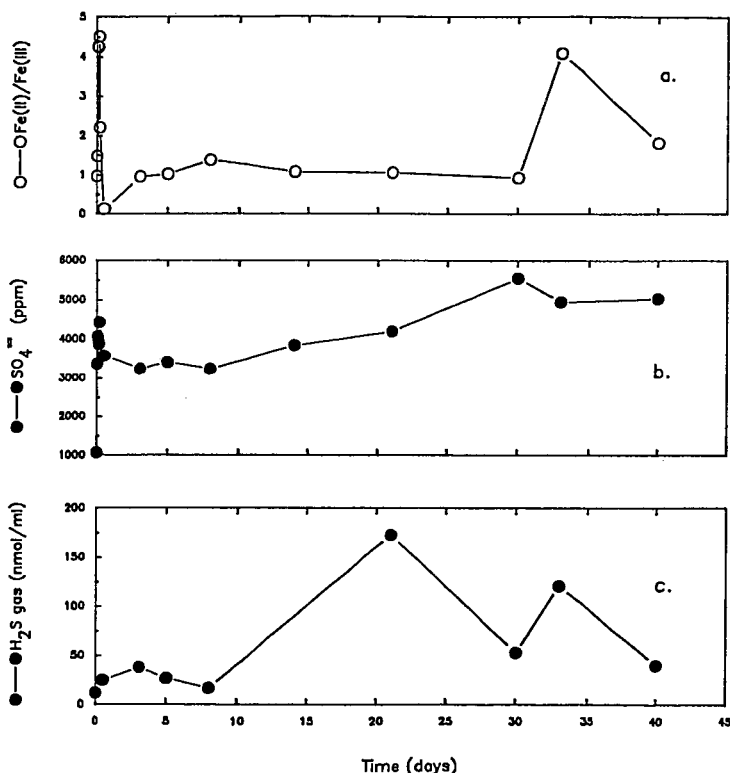
Figure 1. Illinois no. 6 coal (20 g) weathered in an acidic, aqueous suspension (1L, 0.04 M H_2SO_4) gave the above profiles for iron and sulfate concentrations in solution and H_2S generation upon bioassay. a) For the Fe(II)/Fe(III) ratio, total and ferrous iron concentrations were determined spectrophotometrically (1,10 phenanthroline reaction, absorbance 510nm). Ferric iron was determined by difference. b) Concentration of SO_4 was determined turbidimetrically ($BaSO_4$, absorbance 340nm). c) In the bioassay, gaseous H_2S was determined through gas chromatographic analysis of head gas from batch cultures of *P. furiosus* on timepoint samples of weathered coal (0.25g). Values of H_2S are normalized by gas injection volume.

Note: The amount of reducible sulfur available to the microorganisms changes during the course of weathering suggesting a change in the distribution of oxidation products.